

Original article

**INFLUENCE OF SOME PROCESS AND FORMULATION
VARIABLES ON THE FORMATION OF POLYMERIC
MICROSPHERES OF ITRACONAZOLE**

POLİMERİK İTRAKONAZOL MİKROKÜRELERİNİN OLUŞUMU ÜZERİNE BAZI
İŞLEM VE FORMÜLASYON DEĞİŞKENLERİNİN ETKİSİ

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ABSTRACT

Itraconazole is a hydrophobic, antifungal drug. In conventional therapy, itraconazole is normally prescribed as an oral formulation at doses of 200 mg per day and 400 mg per day for serious infections, which can lead to potential toxicity. More efficient means of delivery are needed in order to reduce the toxicity of the drug with the help of reducing often drug dosing. In order to achieve this purpose modified release microspheres of itraconazole was prepared for oral administration. Itraconazole was encapsulated within ethyl cellulose microspheres by water-in-oil-in-water (w/o/w) multiple emulsion technique. Dichloromethane was used as the primary solvent for itraconazole and the polymer. Tween 80 was used as a surfactant for stabilizing external water phase and Span 80 was used for stabilizing the oil phase. The influence of various processing variables like stirring speed and production method on particle size of the microspheres were investigated. The prepared microspheres were evaluated by microscopy, yield of microspheres, particle size analysis, drug entrapment efficiency and drug release studies. Microscopy results revealed spherical nature and smooth surface morphology of the microspheres. The size of microspheres varied between 45-230 µm, and as high as 75.00 % drug entrapment efficiency was obtained depending upon the processing variables. Dissolution studies were carried out for 8 hours at pH 7.4 phosphate buffer solution (PBS) and total amount of drug released from microspheres which were prepared with a rotation speed of 900 rpm and w/o/w multiple emulsion method (F33 and F43) were between 61.70-68.43 %, and

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microspheres that were produced with a lower stirring speed (700 rpm) and o/w emulsion method (F13), drug release was found to be 46.37 % .

Key words: *Microspheres, Itraconazole, Water-in-oil-in-water multiple emulsion technique, Span 80, Tween 80, Ethyl cellulose, Sodium alginate*

ÖZET

İtrakonazol, hidrofobik antifungal etkili bir etkin maddedir. İtrakonazol geleneksel tedavide, oral formülasyonları şeklinde 200 mg/gün dozda, ciddi enfeksiyonlarda ise 400 mg/gün dozda kullanılmaktadır ki bu doz potansiyel toksisiteye yol açabilir. Sık dozlamayı, böylelikle toksisiteyi düşürebilecek daha etkili ilaç taşıyıcı sistemlere ihtiyaç vardır. Bu amaçla itrakonazolun modifiye salım yapan mikroküreleri oral yoldan uygulanmak üzere hazırlanmıştır. İtrakonazol su içinde yağ içinde su (s/y/s) çoklu emülsiyon tekniği ile etil selüloz mikroküreleri içine enkapsüle edilmiştir. Polimer ve itrakonazolun birincil çözücüsü olarak diklorometan kullanılmıştır. Tween 80 dış su fazını, Span 80 ise yağ fazını stabilize etmek üzere yüzey etkin madde olarak kullanılmıştır. Partikül büyüklüğü üzerine karıştırma hızı ve üretim metodu gibi bazı işlem parametrelerinin etkisi araştırılmıştır. Hazırlanan mikroküreler, mikroskop analizi, işlem etkinliği, partikül büyüklüğü analizi, etkin madde yükleme oranı ve mikrokürelerden etkin madde çıkışı ile değerlendirilmiştir. Mikroskop analizi sonuçları mikrokürelerin düzgün bir yüzeye ve küresel bir yapıya sahip olduklarını göstermektedir. Mikrokürelerin büyüklüğü 45-230 µm arasında değişmekte ve işlem parametrelerine göre en yüksek % 75.00 oranında etkin madde yükleme kapasitesine erişilebilmektedir. Çözünme hızı çalışmaları 8 saat süreyle pH 7.4 fosfat tamponu (PBS) içinde yapılmış ve mikrokürelerden toplam etkin madde çıkışının 900 rpm karıştırma hızı ve s/y/s çoklu emülsiyon tekniği ile üretilen (F33 ve F43) mikrokürelerde % 61.70-68.43 arasında iken daha düşük karıştırma hızında (700 rpm) ve y/s tekniği ile üretilen mikrokürelerde % 46.37 oranında olduğu tespit edilmiştir.

Anahtar kelimeler: *Mikroküreler, İtrakonazol, Su içinde yağ içinde su (s/y/s) çoklu emülsiyon tekniği, Span 80, Tween 80, Etil selüloz, Sodyum aljinat*

INTRODUCTION

The prevalence of bacterial, viral or fungal infectious diseases increases each year because they can transmit person to person very easily. Quick and influential treatments are required to prevent spreading of disease to organs and deaths. Itraconazole (ITZ) is a broad spectrum triazole antifungal that is used in treating and preventing fungal infections which are caused by *Aspergillus* and *Candida*. In conventional therapy, ITZ is normally prescribed as an oral formulation at dosages of 200 mg per day and 400 mg per day for serious infections, which can lead to potential toxicity. More efficient means of delivery is needed in order to reduce the toxicity of the drug with the help of reducing often drug dosing (1). Moreover, a multiunit system spreads

over a large area of the absorbing mucosa and prevents exposure to a high drug concentration, when compared to single unit dosage form on chronic dosing (2). In order to achieve this purpose modified release microspheres of ITZ was prepared. The microencapsulation process in which the removal of the hydrophobic polymer solvent is achieved by evaporation has widely been reported in recent years. The technique of emulsion solvent evaporation offers a versatile, easy, and practical method for the manufacture of microspheres because it requires only mild conditions such as ambient temperature and constant stirring. Although the solvent evaporation method seems to involve a relatively simple process, final product characteristics depends mainly on the formulation and process variables. The water-in-oil (w/o) emulsification process has been developed for the encapsulation of highly water soluble drugs. However, an important drawback of using an oil external phase is cleaning up the final product. Unlike w/o emulsification method, water-in-oil-in-water (w/o/w) emulsion solvent evaporation technique proves much more effective when the aqueous solubility of drug is high (>900 mg/ml) and partitioning into the organic phase is unfavorable. Microspheres containing *hydrophilic drugs* have been prepared using different polymers such as poly(lactide-co-glycolide) (PLGA), Eudragit RS (3,4). In literature there are only very few examples about the modification of w/o/w emulsion solvent evaporation for the successful application of hydrophobic drugs into polymeric microspheres (2). Ethyl cellulose, a non-toxic, stable, inert, hydrophobic polymer, is an extensively studied biocompatible polymer for the modified release of pharmaceuticals. However, it has been little searched for either conventional or modified w/o/w technology except Maiti et al. (2). In this study, efforts have been made to incorporate extremely low water soluble drug; itraconazole into polymeric microspheres by w/o/w emulsion solvent evaporation technique and with the help of this method is to improve the trapping efficiency of itraconazole into ethyl cellulose microspheres by alginate facilitated w/o/w emulsion solvent evaporation technique and to investigate the effect of processing variables which are likely to influence the properties of microspheres such as surface morphology, drug entrapment efficiency, in vitro drug release behaviors to achieve to reduce often drug dosing in antifungal itraconazole therapy.

MATERIALS AND METHOD

Materials

ITZ was a gift from Nobel İlaç, İstanbul, Turkey. Ethyl cellulose (18–22 cps viscosity grade) Fluka Biochemika. Sodium alginate (Manucol LKX; 60-170 cP; Keltone LCVR; 100-300 cP),

FMC Bio Polymer. Span 80, Tween 80, dichloromethane were supplied by Merck Ltd. All other reagents obtained commercially were of pharmaceutical grade and used as received.

Methods

Preparation of conventional ITZ microspheres

ITZ (60 mg) and ethyl cellulose (500 mg) were dissolved in 25 ml of dichloromethane. The resultant polymeric solution was then added slowly into 140 ml water containing 0.6% (w/v) Tween 80 with continuous agitation to form o/w type emulsion. The agitation was continued for 2 h to evaporate the organic solvent. Two different agitation speeds were tested 700 and 900 rpm. Finally, the microspheres were filtered off, washed with cold double distilled water (3×100 ml), and dried to constant weight (2).

Preparation of microspheres by modified multiple emulsion technique

The microspheres were prepared by alginate facilitated w/o/w emulsion solvent evaporation technique. Accurately weighed amount of ITZ (5 mg/ml of internal aqueous phase volume) was uniformly dispersed in 8 ml of 2% (w/v) aqueous sodium alginate solution (pH 6.0). The dispersion was added slowly (0.15 ml/s) into 25 ml 2% (w/v) solution of ethyl cellulose in dichloromethane containing 0.5% (w/v) Span 80 and for 5 min at 900 rpm. The resulting water-in-oil (w/o) emulsion was then transferred (2.5 ml/s) into 100 ml of water containing 0.6% (w/v) Tween 80 with continuous mechanical stirring at 700 rpm (or 900 rpm) to form w/o/w type emulsion at room temperature. The stirring was continued for a period of 1.5 h to allow evaporation of the organic solvent. The resulting microspheres were separated by filtration. The remaining loose drug on the surface of the microspheres was removed by washing with cold double distilled water (3×100 ml) and finally oven-dried at 40–50°C for 24 h (2).

Characterization of ITZ microspheres

Surface morphology of microspheres

The shape and empirical surface morphology of the ITZ-loaded ethyl cellulose microspheres were investigated using an optic microscope (Olympus BX 50, Turkey) (5).

Particle size analysis

Particle size of the microspheres was done by sieving method. Mini sieve set ranging 16 to 120 meshes were arranged in a nest. A weighed amount of samples were placed on the top and the

sieve set was shaken for 10 min. The samples retained on each sieve were collected and weighed. The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula (2,6).

Mean particle size = \sum (mean particle size of the fraction x weight fraction) / \sum (weight fraction)

Yield of microspheres

The yield of the microspheres was calculated as a percentage of the total amounts of polymers and drug employed during the preparation (7).

Determination of drug entrapment efficiency

Accurately weighed, 10 mg of ITZ-loaded ethyl cellulose microspheres was dissolved in 2 ml dichloromethane; 30 ml of pH 7.4 phosphate buffer saline (PBS) solution was added and stirred for 30 min with a magnetic stirrer. The mixture was heated at 50–55°C for 45 min in a thermostatic water bath to remove dichloromethane. After that, the volume was adjusted to 50 ml with fresh PBS solution (pH 7.4) heated at 50–55°C. The solution was cooled, filtered, and an aliquot, after suitable dilution, was analyzed spectrophotometrically at 265 nm. Each experiment was carried out in triplicate (8).

In vitro drug dissolution study

The *in vitro* release of ITZ from ethyl cellulose microspheres was carried out in intestinal fluids using a USP type II dissolution rate test apparatus (Aymes, Turkey). About 50 mg of dried microspheres, accurately weighed, were suspended in 900 ml of pH 7.4 PBS solution. The paddle was rotated at 50 rpm and the temperature was set at 37±0.5°C. At predetermined times, 5 ml sample was withdrawn and replaced with fresh buffer solution. The aliquots were analyzed using a spectrophotometer (UV-VIS, Shimadzu UV 1601) at 265 nm (9).

RESULTS AND DISCUSSION

The present work was aimed to prepare ethyl cellulose microspheres with high entrapment levels of ITZ by alginate facilitated w/o/w emulsion solvent evaporation technique.

Effect of stirring speed

The impact of agitation on the production of ITZ loaded microspheres was studied. The change in agitation speed from 700 to 900 rpm was associated with increase in percentage

entrapment efficiency (EE) of the microspheres in o/w method but in w/o/w modified multiple emulsion technique, stirring speed seems to have a minor effect on the EE (Table 1 & Figure 1).

Table 1. Formulation parameters of ITZ microspheres

Formulations	Method	Sodium alginate *(viscosity range of 1% solution)	Stirring rate (rpm)	%EE	Yield (%)	Mean particle size (µm)
F11	o/w	-	700	47.19±2	70.66±1.5	230±18
F13	o/w	-	900	57.24±3	78.39±2.0	60±11
F21	w/o/w	-	700	48.71±1	74.32±1.8	230±23
F23	w/o/w	-	900	49.50±2	77.76±1.5	80±19
F31	w/o/w	60-170 cP*	700	75.00±2	75.89±1.0	100±14
F33	w/o/w	60-170 cP*	900	74.58±1	78.44±2.0	50±17
F41	w/o/w	100-300 cP*	700	72.00±1	73.66±2.0	70±15
F43	w/o/w	100-300 cP*	900	72.18±2	75.76±1.5	45±11

Changing the stirring speed also seems to influence the mean particle size of the microspheres as shown in Table 1, when the speed was increased, particle size of the microspheres was decreased. The increased stress generated in the emulsion tended to divide the droplets of the emulsion and finally resulted in small particles. However, the microspheres maintained their spherical shape and smooth surface characteristics at low stirring speed; 700 rpm (Table 1). Our results were in accordance with the literature (2).

Yield and entrapment efficiency

The yield and entrapment efficiency of ITZ loaded ethyl cellulose microspheres of different preparation methods (o/w and w/o/w) are shown in Table 1. It was reported that entrapment efficiency of the drug was dependent on its solubility in the solvents and processing medium (10) and also depends on the physicochemical properties of the drug and polymer (7). While ITZ entrapment efficiency (EE) (%) of the ethyl cellulose microspheres, prepared by conventional o/w emulsion solvent evaporation technique, was 47.19-57.24 %, EE was found to be 72.18-75.00 % when prepared by w/o/w emulsion solvent evaporation technique (Table 1). Also, it was observed that the viscosity of the sodium alginate has a role in the entrapment efficiency. EE was determined to be 74.58-75.00 % when sodium alginate having a viscosity of 60-170 cP was used in the formulation (F31&F33) but the EE was found to be 72.00 % and 72.18 % (F41&F43), when sodium alginate having a viscosity of 100-300 cP was chosen.

Surface morphology of microspheres

The shape and surface morphology of the ITZ-loaded ethyl cellulose microspheres can be seen in Figures 1&2. In Figure 1, microspheres prepared with a rotation speed of 700 rpm can be seen.

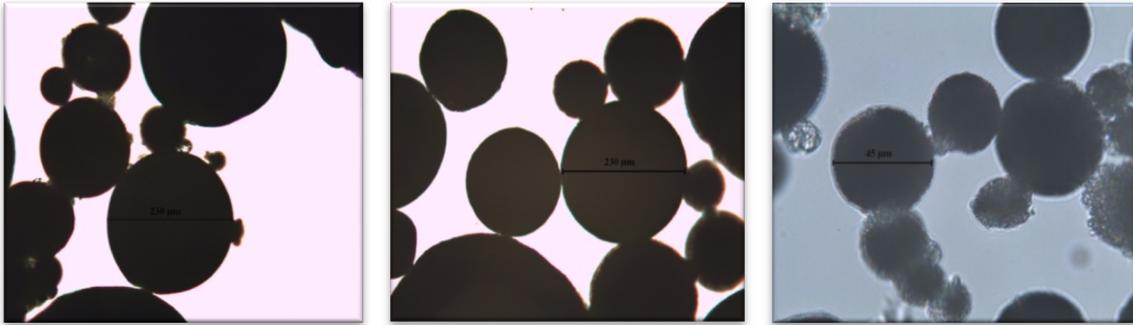


Figure 1. The shape, size and surface morphology of the ITZ-loaded ethyl cellulose microspheres prepared with a rotation speed of 700 rpm (Formulations F11, F31 and F41 respectively).

In Figure 2, microspheres prepared with a rotation speed of 900 rpm can be seen. All microspheres were spherical and smooth as far as can be determined with an optic microscope.



Figure 2. The shape, size and surface morphology of the ITZ-loaded ethyl cellulose microspheres prepared with a rotation speed of 900 rpm (Formulations F13 and F33, F43 respectively).

In vitro drug release

The *in vitro* drug release profile of the microspheres is shown in Figure 3. The variation in stirring speed and preparation method of microspheres affected ITZ release from ethyl cellulose microspheres in pH 7.4 PBS (n=3). Microspheres which were

prepared with a 900 rpm stirring speed (F33 and F43) and having smaller particle sizes, ITZ release was found to be higher which might be related with the increase in the total surface area of the microspheres. In vitro drug release studies also showed that, ITZ release from ethyl cellulose microspheres was also related with the preparation method, microspheres prepared with modified multiple emulsion technique (F23, F33 and F43) had a higher drug release than the microspheres prepared with o/w method (F13) (Figure 3). Dissolution studies were carried out for 8 hours at pH 7.4 and total amount of drug released from microspheres which were prepared with a rotation speed of 900 rpm and w/o/w multiple emulsion method (F33 and F43) were between 61.70-68.43 %, but microspheres that were produced with a lower stirring speed (700 rpm) and o/w emulsion method (F13), drug release was found to be 46.37 % .

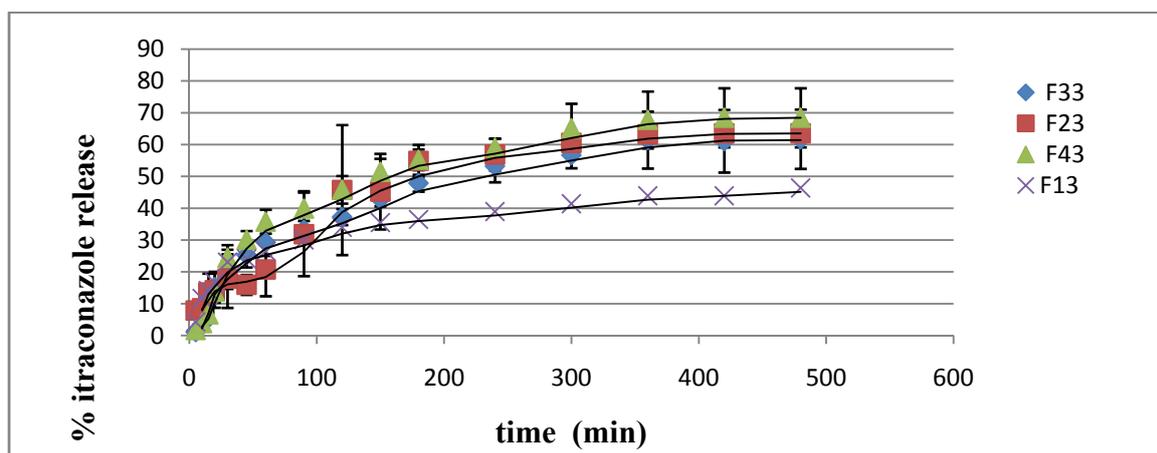


Figure 3. ITZ release from ethyl cellulose microspheres

CONCLUSION

This study suggested that the use of sodium alginate solution as an internal phase in conventional double emulsion solvent evaporation method may be an alternative approach for the successful incorporation of poorly water soluble drugs in ethyl cellulose microspheres. This investigation has also provided an understanding of the effects of some processing parameters on the characteristics of the microspheres. Proper control of the processing variables allowed the entrapment efficiency of as high as 75.00 % itraconazole in the microspheres and enabled the preparation of smooth, spherical microspheres having size range between 45-230 μm . The microspheres were able to provide drug release for an extended period of time (8 hours) in pH 7.4 PBS solution with a drug release of 61.70-68.43% (F33 and F43).

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